The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour

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Abstract

Current host-specificity testing procedures in weed biological control fail to acknowledge behavioural phenomena that have the potential to impact on the interpretation of test results. I review current procedures and propose a new procedure that takes into account mechanisms known to underlie the behavioural process of host-plant finding and acceptance. The new procedure minimizes the likelihood of results that may lead to the rejection of safe insect species or, conversely, could lead to the release of potentially unsafe insect species for weed biological control. In addition, the period of time which elapses between the acceptance of the target weed and lower-ranked plant species, by candidate biological control agents, can be determined. I suggest that this period may be as important a measure of specificity as the actual number of plant species susceptible to attack.

Introduction

The objective of host-specificity testing in weed biocontrol is to determine whether candidate biological control agents will attack non-target plant species once released in new regions. Current host-specificity tests estimate field host-plant range by determining the susceptibility of a selection of taxonomically closely related plant species to the candidate agent under glasshouse or laboratory conditions (Harley & Forno, 1992).

Host-specificity test results for candidate weed biological control agents are often ambiguous (Dunn, 1978; Cullen, 1990). Some weed biological control practitioners consider that the tests often over-estimate host range. This may lead to the rejection of candidate agents that would be adequately specific under field conditions (see Dunn, 1978; Wapshere, 1989; Cullen, 1990). In contrast, other biologists are becoming increasingly concerned about the safety and potential negative environmental impact of biological control agents (see Harris, 1990; Howarth, 1991; Marohasy, 1996; Simberloff & Stiling, 1996).

In their real world environments, phytophagous insects employ a complex behavioural sequence to find and then determine the suitability of potential host plants. Yet an analysis of the mechanisms underlying the process of host-plant finding and acceptance is not usually included in the design or interpretation of host-specificity tests for weed biological control. In this paper I review host-specificity testing procedures used in weed biological control. I then focus on behavioural phenomena that may give ambiguous or false test results. A new three-tiered procedure for establishing the relative acceptability of test-plant species to candidate biocontrol agents is proposed. The new procedure would minimize the possibility of false results and enable the determination of the length of the discrimination phase (sensu Singer et al., 1992) as well as host-plant range.

Traditionally host-specificity tests determine either the propensity of adults and immatures to feed and oviposit on test-plant species (e.g. Marohasy, 1994; Heard & Forno, 1996), or the degree to which a test-plant species can support pre-reproductive and reproductive development (e.g. McFadyen & Marohasy, 1990; DeLoach & Cuda, 1994).

In this paper I consider only host-specificity tests concerned with feeding and oviposition. I accept that determining whether an insect can complete development on test-plant species, and/or comparing development times on test-plant species, may be the most practical approach for the host-specificity testing of particular candidate biological control agents (Cullen, 1990). However, a discussion of the design and interpretation of such tests is beyond the scope of this paper.

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History of the Development of Host Specificity Testing Procedures for Weed Biological Control

Many early workers believed the field host range of an insect species in its country of origin, as observed by the surveying entomologist, was the best indicator of host-specificity (Dodd, 1940). Early resistance to the concept of experimentally testing host-plant range existed, at least in Australia: "... the plan of endeavouring to prove by experiments whether insects could develop in plants unrelated to their natural hosts was a new procedure, at least in so far as it concerned the introduction of insects from one country to another. The consensus of scientific opinion was not favourable and could see little value in the proposed starvation tests, arguing that natural host restriction in America (the country of origin) must be the all important factor." (Dodd, 1940, p.51). However, there was community and political pressure for experiments. The experiments became known as host-specificity tests and were usually undertaken in cages (Dodd, 1940).

No-choice or sequential host-specificity tests of varying duration became the accepted methodology for the determination of host-specificity until the late 1960s (Harris & Zwolfer, 1968). In no-choice tests, candidate agents are confined for varying periods of time usually with only one test plant species and without the target weed (e.g. Harris, 1963; Frick & Andres, 1967). Sequential tests involve the sequential presentation of a series of test plants, each in a no-choice situation. In sequential tests each plant species is usually exposed to the candidate agent for a relatively short time (e.g. Bennett, 1967).

In the late 1960s, Harley (1969) advocated choice as opposed to no-choice tests for host-specificity testing. In choice tests, an array of test-plant species is exposed to the candidate agent for varying periods of time in the presence of the target weed. Advocates argued that choice tests are a more "natural test" of host range, as the target weed will be present in the field (Harley, 1969; Cullen, 1990). There may be fewer incidences of feeding on test-plant species in choice tests (Table 1). However, results from cage tests, whether choice or no-choice, are often ambiguous (Cullen, 1990). In particular, "cages place restrictions on an insect's natural host-finding behaviour, often leading to its selection of unnatural hosts" (Clement & Cristofaro, 1995).

In the development of all the above-mentioned test procedures, limited, if any, formal consideration has been given to mechanisms underlying the behavioural process of host-plant finding and acceptance. I will show that all the above-mentioned tests may give false results.

**Table 1.** Examples of discrepancies between choice and no-choice test results.

<table>
<thead>
<tr>
<th>Insect species</th>
<th>No-choice tests</th>
<th>Choice tests</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calophasia luna (Hufnagel) (Lep.: Noctuidae)</td>
<td>Larvae fed on, and could complete development on snapdragon Antirrhinum majus (Scrophulariaceae).</td>
<td>No feeding on A. majus.</td>
<td>Harris, 1963</td>
</tr>
<tr>
<td>Microlarinus lareynii (Duval) M. lypriformis (Wollaston) (Col.: Curculionidae)</td>
<td>Adults fed on 27 and 16 plant species in 17 and 12 families, respectively. However oogenesis only occurred when Tribulus terrestris (Zygophyllaceae) or other closely related genera were present.</td>
<td>Fed only on weed and field host T. terrestris.</td>
<td>Andres &amp; Angalet, 1963</td>
</tr>
<tr>
<td>Uroplata girardi Pic (Col.: Chrysomelidae)</td>
<td>Adults oviposited and fed on Lippia alba, Tectona grandis, Lantana montevidensis and Lantana trifolia (all Verbenaceae).</td>
<td>Fed and oviposited exclusively on target weed Lantana camara.</td>
<td>Bennett &amp; Maraj, 1967</td>
</tr>
<tr>
<td>Leptinotarsa decemlineata (Say) (Col.: Chrysomelidae)</td>
<td>Fed on Capsella bursa-pastoris (Brassicaceae) and Galinsoga parviflora, Lactuca scariola and L. sativa var. romana (all Asteraceae).</td>
<td>Did not feed on the 4 species.</td>
<td>Hsiao &amp; Fraenkel, 1968</td>
</tr>
<tr>
<td>Neohydronomus affinis Hustache. (Col: Curculionidae)</td>
<td>Fed on Orontium aquaticum (Araceae); fed and oviposited on Lemna minor, Spirodela punctata and S. polyrhiza (all Lemnaceae), Limnobium spongia (Hydrocharitaceae), Azolla caroliniana (Azollaceae) and Salvinia minima (Salviniaeae).</td>
<td>Fed and oviposited exclusively on weed Pistia stratiotes (Araceae).</td>
<td>Thompson &amp; Habeck, 1989</td>
</tr>
</tbody>
</table>

**Behavioural Phenomena and Host Specificity Testing**

Some species of insect, and in particular many weed biological control agents, exhibit extreme host specificity and are unlikely to attack even closely related plant species under any conditions. However, the process of host-plant finding and acceptance in most insect species is more labile and both the insect’s endogenous condition and the test arena may...
A behaviourally based host-specificity test should indicate whether a plant species is susceptible to feeding or oviposition by a candidate biological control agent under any possible field conditions. I distinguish two types of false results. When feeding or oviposition occurs on a test-plant species, which would not be attacked in the field, a false positive occurs. A false negative occurs when a test indicates a plant species is outside the host range of the insect species, when in reality it might be attacked in the field. False positives are potentially expensive as they may result in the rejection of an agent that would be safe for release. False negatives are potentially dangerous, as they could lead to the release of an agent, which, under some circumstances, will damage non-target plant species. Whether a testing procedure gives a false negative or false positive result will obviously depend on the characteristics of the particular insect species being tested. However, some phenomena are more likely to be induced under some host-specificity testing procedures than others (Table 2). Five behavioural phenomena most likely to affect a test result are discussed below and their implications for particular host-specificity testing procedures shown in Table 2. The phenomena are listed in likely order of importance.

### Time-dependent changes in responsiveness to plant species

Papaj & Rausher (1983) have proposed the term “time-dependent changes” to refer to reversible changes in responsiveness to food- and oviposition-site deprivation. The change in responsiveness is a result of a change in the threshold necessary to elicit a positive response in the relevant decision-making centres of the central nervous system, and/or a change in the responsiveness of sensory receptors to excitatory stimuli (see Bernays & Chapman, 1994).

Current theories indicate that an insect will feed or oviposit on a plant species if the net level of excitation generated by that plant exceeds some internal threshold that is specific for that individual at that time. The threshold necessary to elicit a positive response by an insect is generally high immediately following a period of feeding or oviposition and declines with time thereafter (Schoonhoven, 1987; Singer et al., 1992). As a consequence, an insect deprived of the opportunity to feed or oviposit (e.g. on its principal host plant) will show an increased sensitivity to stimulatory plant-based cues and as a result may accept plants that would be rejected after shorter periods of deprivation (Wiklund, 1981; Fitt, 1986; Schoonhoven, 1987).

In open-field and cage-choice tests, insects have unlimited access to the target weed and thus may never become responsive to ‘less excitatory’ lower-ranked plant species. As a consequence, a false negative result is likely to be recorded (Table 2). In no-choice tests of adequate duration, a false negative is much less likely. Without access to the target weed the candidate biological control agent may become receptive to lower-ranked host plants. However, if several lower-ranked host plants are simultaneously presented, only the most acceptable (i.e. highest ranked) lower-ranked host plants may be attacked.

### Pre-alighting cues bypassed

Host-plant finding and acceptance in the field often involves a number of different behaviours (Miller & Stricker, 1984). Each behaviour may be mediated by a different set of sensory cues (Bernays & Chapman, 1994). For example, habitat-specific olfactory cues may be perceived from a distance. Once within the habitat, visual cues may then be important in distinguishing colours and shapes of plant parts used for feeding and/or oviposition. Upon making contact with a target weed, a plant species will show an increased sensitivity to stimulatory plant-based cues and as a result may accept plants that would be rejected after shorter periods of deprivation.

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**Table 2. Behavioural phenomena that may cause false negative and false positive results under particular host specificity testing regimes. False negatives = no attack in test, but potential for attack under field conditions. False positive = attack in test, but no potential for attack under field conditions.**

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Phenomena inducing false negatives</th>
<th>Phenomena inducing false positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open field choice</td>
<td>Unresponsive to lower-ranked potential hosts</td>
<td>Attack on non-hosts positioned close to target weed owing to central excitation</td>
</tr>
<tr>
<td>All cage tests</td>
<td>Escape becomes a priority and insect unresponsive to all plant species</td>
<td>Pre-alighting cues bypassed</td>
</tr>
<tr>
<td>Cage choice</td>
<td>Unresponsive to lower-ranked plant species</td>
<td>Attack on non-hosts positioned close to target weed owing to central excitation</td>
</tr>
<tr>
<td></td>
<td>Central inhibition owing to recent contact with strongly deterrent non-host plants</td>
<td>Associative learning when target weed and non-hosts have some characteristics in common</td>
</tr>
<tr>
<td>Cage no-choice</td>
<td>Unresponsive to lower-ranked plants depending on level of deprivation &amp; testing sequence</td>
<td>Habituation owing to repeated contact with non-hosts.</td>
</tr>
<tr>
<td>Cage sequential</td>
<td>Unresponsive to lower-ranked plants depending on level of deprivation &amp; testing sequence</td>
<td>Volatiles from target weed permeate cage and condense onto non-host foliage</td>
</tr>
</tbody>
</table>

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Cage sequential Unresponsive to lower-ranked plants depending on level of deprivation & testing sequence Associative learning owing to repeated exposure to non-host following exposure to target weed
Experience-dependent changes in responsiveness to plant species

Experience of hosts has been shown to induce changes in the central and/or peripheral nervous system which can influence an insect’s subsequent behaviour towards potential host plants (Jermy, 1987; Papaj & Prokopy, 1989; Szentesi & Jermy, 1990). The effects of experience fall into several categories according to the kind of changes induced and the duration of the effect. The following is a brief summary of these categories of experience and their potential effect on host-specificity test results.

The following four experience-induced phenomena could induce false positive results:

(i) Contact with the target weed may induce a state of central nervous excitation whereby insects feed or oviposit on adjacent plant species or other substrates that would otherwise be rejected (Menzel et al., 1993; Marohasy, 1994). Central excitation is most likely to occur in a cage-choice situation when non-host plants are positioned close to the target weed (Table 2).

(ii) When the stimulatory effect is of a longer duration than central excitation (minutes rather than seconds) the phenomenon is known as sensitization.

(iii) Insects may become habituated to the inhibitory input from non-host plants, to the extent that these cues no longer elicit a response. If during cage tests an insect is confined with such a non-host plant species it might eventually accept the plant following habituation to the inhibitory input elicited by the plant (Table 2). Habituation to deterrents has been demonstrated in several species of phytophagous insects in the context of feeding (Jermy et al., 1982) and oviposition (Traynier, 1979).

(iv) Repeatedly exposing an insect to a host plant species after exposure to a non-host that has similar sensory cues, can result in acceptance of the non-host owing to associative learning (Bernays & Wrubel, 1985; Szentesi & Jermy, 1990). Associative learning could at least theoretically be induced during sequential tests of short duration, if the test insects were placed with the target weed following each sequential test (Table 2).

False negative results could be induced through central inhibition (Table 2). Central inhibition occurs when cues from non-host plants are inhibitory acting as oviposition and feeding deterrents and/or locomotory stimulants. These cues can have the effect of increasing an insect’s acceptance threshold such that normally acceptable plants are rejected (Jermy, 1971). Central inhibition is particularly likely to occur in cages when non-host plants are positioned close to otherwise acceptable plant species or when the cage is perfused with a strong inhibitory odour (Table 2).

The experience-induced phenomena known as induction of preference may also affect a test result. Induction of preference affects the threshold of acceptance of plant species such that previously experienced species become relatively more acceptable than other plant species (Szentesi & Jermy, 1990). However, induction of preference is more likely to be an effect of the insect’s experience prior to the host specificity test than an effect of the particular testing procedure. The strongest inductive effects have been seen in insects that have fed on lower-ranked plant species resulting in a preference for these plant species over the otherwise higher ranked hosts (Jermy et al., 1968; Ma, 1972; Szentesi & Jermy, 1990).

Absorption of volatile kairomones

If excitatory volatile chemicals from the target weed permeated the cage and condensed onto test plants, then the candidate biological control agent may feed and oviposit on the affected plant species (e.g. Jayanth et al., 1993). Choice-cage tests, where test-plant species are often placed near the target weed, would be most likely to induce this type of false positive result (Table 2).

Indiscriminate behaviour

The cage environment may be inhibitory owing, for example, to low light intensities. As a result, feeding and oviposition may be delayed until the insect is in an extreme state of food- and/or oviposition-site deprivation. Alternatively, when insects are placed in cages, dispersal and departure behaviour may be inhibited and as a consequence the normal process of host-plant finding and acceptance may appear to go awry (Dunn, 1978; Cullen, 1990). Two scenarios are possible:

(i) An insect may ‘dump’ its eggs on normally unacceptable plant species in response to the perception that death is imminent (Roitberg et al., 1993), potentially inducing a false positive result (Table 2).

(ii) The ‘escape response’ may override other behaviours and the insect may become unresponsive to all plant species, potentially inducing a false negative result (Table 2).

Proposed New Procedure for Host Specificity Testing

The three-tiered procedure proposed in this section aims to minimize the likelihood of false positive and false negative results and determine the relative acceptability of test plant species. The procedure is untried and will probably be found to have limitations additional to those already recognized.
Nevertheless, it will provide a starting point for a more theoretical approach to the design and interpretation of behaviourally based host-specificity tests for potential weed biological control agents. I first outline a procedure for testing both feeding and oviposition as applied to insects whose biology is such that a mobile female is responsible for host plant location. Modifications to this system will be needed when this is not the case, and are discussed at the end of this section.

The location of a plant species is the first stage in the behavioural sequence leading to attack on a novel plant. Only lifestages that have the potential to disperse, either actively or passively, have the potential to find new plants. I therefore propose that only these lifestages be tested, or at least that these lifestages be tested first. Lifestages with poor mobility may make behavioural-based decisions whether or not to feed. However, in a ‘real world’ situation such decisions would be restricted to plant species that had been located and accepted by the mobile lifestage(s).

Insects should be kept with the target weed prior to testing. In some insect species the presence of the principal host-plant is necessary for reproductive development because reproductive development may be supported by nutrients obtained through adult feeding (Slansky, 1980); the rate of oocyte maturation may be enhanced by host cues (Andres & Angalet, 1963); or mating may be less likely, or does not occur in the absence of the principal host plant (McNeil, 1991). In these insect species, false negative results may be recorded if the insect does not have access to its principal host plant prior to testing. It could be argued that species that require the presence of the target weed for reproductive development are unlikely to damage significantly non-target plant species in the field. However, this will depend on the spatial arrangement of susceptible plant species. For example, the weevil *Microlarinus larregnii* (Duval) (Col.: Curculionidae) only produce eggs in the presence of its principal host-plant *Tribulus terrestris* L. (Zygophyllaceae) (Andres & Angalet, 1963). Yet *M. larregnii* has been found attacking *Kallstroemia grandiflora* Torr. (Zygophyllaceae) in the field (Turner, 1985).

When insects have previous experience of a particular plant species, there is always the risk of a false result owing to induction of preference. However, significant effects of induction of preference appear to be most common when naive insects have first experienced lower-ranked plants (see Section: Experience-dependent changes in responsiveness to plant species).

**Stage 1: Determining whether any test plant species are susceptible to attack**

To determine whether any test-plant species are susceptible to attack under a range of possible field conditions, I propose tests first should be undertaken in the absence of the target weed. Representatives of the various test-plant species can be exposed individually, or in various combinations. Placing the test plants together in a cage would enable species to be tested simultaneously, reducing the number of candidate biological control agents needed for testing, and the number of cages required. However, placing all the test plants together creates a theoretical possibility of a false negative result owing to central inhibition. This phenomenon has been little studied (Jermy, 1971) and is difficult to demonstrate without relatively complex behavioural experiments. However, by exposing test plants in different combinations in cages it would be possible to demonstrate that it is highly unlikely this phenomenon is influencing the results.

Controls should include specimens of the target weed exposed concurrently in a separate cage, using a cohort of insects from the same origin as those being tested. The control is necessary to ensure insects are in a physiological state such that feeding or oviposition would occur readily on the principal host plant. The tests should be of adequate duration such that insects would experience oviposition site and feeding deprivation if no acceptable plant species were present. Preliminary experiments may be necessary to determine this period which may be as short as several hours (e.g. coccinopyiid fly) or several days (e.g. chrysomelid beetle).

If feeding or oviposition do not occur on test plants, we can conclude that the candidate biological control agent will not attack the test-plant species after release. If some feeding or oviposition occurs on any species, other test plants included in combination with that species may be susceptible to attack but are lower ranked hosts.

**Stage 2: Determining the rank order of susceptible plant species**

The extent of oviposition and feeding on a plant species in a field situation will depend on the acceptability of the plant species relative to other species and the target weed (Wiklund, 1981; Singer et al., 1989). Establishing rank order, also known as preference rank (Thompson, 1988; Jallow & Zalacki, 1996), is a first step towards establishing relative acceptability and thus the likelihood of a lower-ranked plant species being attacked in the field.

The rank order of 13 plant species for a Swedish population of *Papilio machaon* L. (*Papilionidae*) was determined by presenting test-plant species in large cages in a sequential pattern (Wiklund, 1975). The highest-ranked plant species (determined from field data) were presented on the first day. The number of eggs deposited on each plant species was counted at the end of that day. The following day the plant species that had received the largest number of eggs was replaced by a new test-plant species. This process was continued until all test-plant species were within the arena. At this point the number of plants within the cage began to decline as the plant species that received the most eggs continued to be removed. If no eggs were laid on plants during any one day, females were again presented with the previous day’s plants the next day (Wiklund, 1981).

I propose a similar methodology to determine the rank order of test-plant species to candidate biological control agents in weed biological control. Plant species should be first assigned a provisional rank order based on:

(i) number of eggs laid or extent of feeding on plant species in Stage 1;
(ii) taxonomic, chemical and morphological similarity;
(iii) data from field surveys in the candidate agent’s native range; and
(iv) museum records and literature searches.

On day 1, specimens of the target weed should be placed in cages with test-plant species predicted to be most highly ranked. After a pre-determined period of adequate duration (see Stage 1) the plant species that receives the most eggs or feeding damage (presumably the target weed after the first period) should be removed and the presumed next most highly-ranked plant species (of those not already in the arena) introduced. The process should be continued until there is no feeding or oviposition on any plant species. There should be a control in which the candidate agent has access to only the target weed to ensure insects are in a physiological...
state such that feeding or oviposition would occur readily on the principal host plant. The tests should be replicated.

By removing the highest-ranked plant at the end of each period, the problem of false negative results owing to unresponsiveness to lower-ranked plant species is overcome. Plant species can be ranked from most acceptable (those removed first) to less acceptable (those removed subsequently). Species that receive no feeding or oviposition can be considered immune to attack. The presence of a non-host plant species with deterrent or inhibitory qualities in the cages could induce a false negative result through central inhibition. If there is no feeding or oviposition initially in the presence of the target weed, then central inhibition could be hypothesized and plant species removed until the species inducing the effect is identified. If there is no feeding or oviposition during any one period following the introduction of a plant; then the previous period’s array of plants should be reintroduced. If feeding or oviposition resumes, then we can assume the new plant had inhibitory qualities.

Central excitation, indiscriminate behaviour, and sensitization may also occur, potentially inducing a false positive result. However, because the plant species that is most stimulatory (i.e. receives most feeding or oviposition) is removed at the end of each period, any marked decrease in the level of attack on previously adjacent plant species (because of the previous effects of central excitation or sensitization) should be evident. As non-hosts may remain in the cage for the duration of the test a false positive could occur through habituation. However, even in a worst case scenario, feeding or oviposition on these plant species would be reduced relative to feeding and oviposition on more acceptable species. These plant species may thus falsely be considered low-ranked hosts, rather than non-hosts.

If cages are adequately large with adequate ventilation and lighting, false positives should not result owing to the by-passing of pre-alighting cues or the condensation of volatiles from the target weed or other plant species. However, caged insects may show other behaviours (e.g. escape response) that override behaviours associated with host-plant finding and acceptance (Table 2). If this is the case, then open-field specificity tests may be necessary. However, in open-field tests the potential for false negative results owing to unresponsiveness to lower-ranked plant species will always be high (Table 2).

**Stage 3: Quantifying the length of the discrimination phase**

In weed biological control, the term specificity usually refers to the range of plant species susceptible to attack by a biological control agent. Host specific control agents attack only the target weed and perhaps a limited number of closely-related plant species. However, Singer et al. (1992) defines specificity as the time over which a female accepts one plant while the lower-ranked plant is rejected. An individual female that spends a long time in this discrimination phase (i.e. searching for its principal host plant) is classified as very specific irrespective of its potential host-plant range (Singer et al., 1992; Jallow & Zalucki, 1996).

The discrimination phase in monophagous insects is thus theoretically infinitely long or at least longer than the life of the insect.

If the length of the discrimination phase of a potential biological control agent and the distribution and abundance of potential host-plant species were known, then we could estimate the probability of insects encountering lower-ranked plant species when these plants were susceptible to attack (i.e. after individuals had searched for periods in excess of the discrimination period). In this way we may be able to predict field conditions under which attack may occur on lower-ranked plant species. Predicting the conditions under which field attack may occur, and the likely severity of attack on non-target susceptible plant species must be the ultimate objective of host specificity tests. However, to achieve this we may also need information on the ecology of the species including migration and dispersal patterns under field conditions.

Some of the perceived discrepancies between field and cage plant range may be explainable if the length of the discrimination phase was recognized as well as actual plant range. Candidate biological control agents with long discrimination phases may accept lower-ranked plant species only under particular field conditions. For example, the tingid Teleonemia scrupulosa Stål (Hem: Tingidae) was introduced into many parts of the world beginning in the 1920s for the biological control of the weed Lantana camara (Verbenaceae) including regions where Sesamum indicum L. (Pedaliaceae) is grown. Teleonemia scrupulosa was never recorded attacking S. indicum until the early 1960s when there was a population explosion of T. scrupulosa in Uganda. Severe defoliation of L. camara occurred and T. scrupulosa was recorded for the first time attacking S. indicum growing in close proximity. Potential adult feeding on S. indicum may have been evident had S. indicum been included in the host range testing.

Greathead (1968) referred to the attack on S. indicum by T. scrupulosa as a “temporary break-down of host specificity”. The event perhaps demonstrates that T. scrupulosa has a host range that can include S. indicum, however, T. scrupulosa can nevertheless be considered host specific to L. camara. This is because S. indicum will only be attacked when it grows in close proximity to L. camara which becomes temporarily unavailable (i.e. S. indicum is encountered after a period in excess of the length of the discrimination phase). Also, S. indicum is only accepted for adult feeding and can not support oviposition and nymphal development.

A tethered-insect technique has been successfully used to investigate the length of the discrimination phase with respect to oviposition in the lepidopterans Helicoverpa armigera Hübner (Noctuidae) (Jallow & Zalucki, 1996) and Euphydryas editha L. (Papilionidae) (Singer, 1982). These experiments have demonstrated considerable within- and between-population variation in rank order and length of the discrimination phase. However, the experiments were relatively technically difficult and not all insects are likely to be amenable to this type of experimental manipulation. I propose that the approximate length of the discrimination phase of candidate biological control agents be determined as part of Stage 2. Insects with a low motivation to oviposit or feed (i.e. insects kept with the target weed prior to the test) could be introduced into cages with each new array of plant species. The period to first oviposition or feed would be a measure of the length of the discrimination phase between the target weed and the highest-ranked plant species for the particular array of plant species. It would be important to use insects of the same age and experience for each test.

**Possible modifications for passively dispersed insects**

The three-tiered procedure outlined above is designed primarily for insect species with winged females responsible for host location, and immature stages with limited mobility. When immature stages are also mobile and capable of significant
dispersal, immatures should also be tested using the procedure detailed above (e.g. for scale insects, mealybugs and mites). However, when the life stage responsible for host location is dependent on passive dispersal mechanisms (e.g. wind), failure to encounter some test-plant species is likely to occur under cage conditions unless individuals are placed directly on test-plant species. Under these circumstances, no-choice tests should be undertaken and the proportion of individuals settling on test-plant species could be used to determine rank order. Treatments should be run for different levels of deprivation (i.e. time since last fed on highest-ranked host plant) to avoid the possibility of a false negative result owing to unresponsiveness to lower-ranked hosts. Differences in the proportion settling at the different levels of food deprivation may give an indirect measure of the length of the discrimination phase between the target weed and each lower-ranked test plant species.

Conclusions

The procedure proposed in this paper, and its various possible modifications, is an attempt to introduce a more theoretical and quantifiable approach to host-specificity testing for candidate weed-biological control agents. In their current form, behaviourally based host specificity tests give a relatively crude indication of whether non-target plant species will be susceptible to attack by candidate biological control agents once field released. Biological control practitioners have never significantly refined these tests:

(i) to minimize the possibility of false positive and false negative results;
(ii) to rank less acceptable plant species; and
(iii) to determine the lengths of time agents are likely to search before accepting a lower-ranked plant species by measuring the length of the discrimination phase (i.e. quantify specificity sensu Singer et al., 1992).

Current choice testing procedures risk false negative results, as when insects are tested in the presence of their highest-ranked host plant they may be unresponsive to lower-ranked plant species. False negative results are potentially dangerous as they could lead to the release of insects that under some circumstances will attack non-target plant species. While the damage inflicted to the non-target plant species may be neither significant nor sustained, political repercussions may be substantial (Harris, 1990).

If insects are tested according to the procedure outlined in this paper, we may be able to demonstrate that a candidate biological control agent with a relatively broad host-plant range will only attack non-target plant species under particular conditions because it has a long discrimination phase. The length of the discrimination phase could be quantified. If the spatial distribution of potential host plants was known, different scenarios likely to arise in the field could be explored through computer modelling.

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